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THE COUNCIL FOR TOBACCO RESEARCH—U.S.A., INC.
110 EAST 59TH STREET
NEW YORK, N. Y. 10022
(212) 421-8885

Application for Research Grant
(Use extra pages as needed)

Date: May 8, 1975

1. Principal Investigator (give title and degrees):

Emil R. Unanue, M.D.

2. Institution & address:

Department of Pathology
Harvard Medical School
25 Shattuck Street
Boston, Massachusetts 02115

3. Department(s) where research will be done or collaboration provided:

Department of Pathology

4. Short title of study:

Physiopathology of Normal and Activated Macrophages

5. Proposed starting date: September 1, 1975

6. Estimated time to complete: Three years

7. Brief description of specific research aims:

This application is for continuation of studies on the physiology of macrophages and their role in disease. It focuses on the investigation of biologically active molecules secreted by macrophages: their characterization, regulation, and function in physiological states and disease processes. The experimental project consists of experiments in which macrophages treated in different manners are cultured; the culture fluids are examined and characterized chemically and biologically. Work along the lines described above has been done for the past two years with positive results, some of which have been published. From this initial work, as well as from work of others, it has become quite apparent that macrophages secrete a number of powerful active molecules which have the potential of playing an important regulatory role in *in vivo* processes. Heretofore, the secretion of macrophages had been in great part ignored, yet it may represent as important a function as phagocytosis. So far we have found an inhibitor of cell proliferation and stimulatory molecules that promote lymphocyte proliferation and differentiation; others have found a number of enzymes, such as, for example, a plasminogen activator-like molecule and lysozyme.

This application contains three basic goals: attempts to 1) relate secretion of biologically active molecules with the state of activation and function of the phagocytes; 2) to isolate the molecules; and 3) to define their biological activities. We are concentrating on four activities that appear to promote a heightened immune status: differentiation of thymocytes, differentiation of B lymphocytes, increased helper activity of thymic cells, and chemoattraction.

8. Brief statement of working hypothesis:

Macrophages are cells found throughout the different tissues and endowed with powerful biological functions, mainly as concerns their role in inflammation. Macrophages respond in various ways to external stimuli and are thought capable of regulating a number of cellular functions. The role of macrophages in the lung (alveolar macrophages) in local bacterial resistance is known. Their general response to phlogogenic stimuli, such as inhaled materials, is only partially characterized. In order to properly outline the role of this cell in normal resistance, basic studies on its response are necessary. It is our contention that macrophages play an important regulatory role not only by handling of antigens but also by the elaboration and release of regulatory molecules. These molecules are best released following phagocytosis and serve to focus and increase the specific limb of the immune response, *i.e.*, the lymphocyte response. The biological and chemical characterization of these molecules secreted by macrophages may represent a fundamental and necessary step for our understanding of the function of this cell.

9. Details of experimental design and procedures (append extra pages as necessary)

Please see appended pages.

10. Space and facilities available (when elsewhere than item 2 indicates, state location):

My laboratories are in the Department of Pathology at Harvard Medical School. The Department occupies about 16,000 square feet of space. Common laboratories containing gamma and liquid scintillation counters, spectrophotomer, lyopholizer, etc., are available for all members. Ample animal facilities are found in a building next to the Department. My laboratories occupy about 2,500 square feet of space. They consist of four different, interconnecting rooms with an annex for desk space. The laboratories are fully equipped for tissue culture work, microscopy, and immunochemistry.

11. Additional facilities required:

None

12. Biographical sketches of investigator(s) and other professional personnel (append):

13. Publications: (five most recent and pertinent of investigator(s); append list, and provide reprints if available).

14. First year budget:

A. Salaries (give names or state "to be recruited")	% time	Amount
Professional (give % time of investigator(s) even if no salary requested)		
Emil R. Unanue, M.D.	20	REDACTED
Miguel Stadecker, M.D.	100	
Technical		
To be hired		REDACTED
Part-time animal caretaker		
Sub-Total for A		REDACTED
B. Consumable supplies (by major categories)		
Animals: mice, about 400 to 500 per month; rats; rabbits		$ 9,000
Chemicals and isotopes		2,400
Tissue culture supplies		6,000
Sub-Total for B		$17,400
C. Other expenses (itemize)		
Expenses for animal care and food (about $300 per month)		$ 3,600
Travel to scientific meeting		400
Sub-Total for C		$ 4,000
Running Total of A + B + C		$54,400
D. Permanent equipment (itemize)		
Hewlett Packard 65A Calcultor with programs		$ 930
Sub-Total for D		$ 930
E. Indirect costs (15% of A+B+C)		$ 8,160
Total request		$63,490

15. Estimated future requirements:

	Salaries	Consumable Suppl.	Other Expenses	Permanent Equip.	Indirect Costs	Total
Year 2		18,270	4,200	—	8,667	66,447
Year 3		19,184	4,410	—	9,206	70,582

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16. Other sources of financial support:
List financial support from all sources, including own institution, for this and related research projects.

CURRENTLY ACTIVE

Title of Project	Source (give grant numbers)	Amount	Inclusive Dates
Studies on Antigenic Stimulation (runs to 1978)	N.I.H. AI 10091	$64,017	01/01/75 - 12/31/75
As Co-investigator: *Experimental Cancer Immunology* (Dr. B. Benacerraf is P.I.)	N.I.H. CA 14723	38,611	06/01/75 - 05/31/76
As Co-investigator: *Ultrastructural Immunocytochemistry of Cell Surfaces* (Dr. M. J. Karnovsky is P. I.) (runs to 1978)	N.I.H. AI 10677	38,900	01/01/75 - 12/31/75

PENDING OR PLANNED

Title of Project	Source (give grant numbers)	Amount	Inclusive Dates

It is understood that the investigator and institutional officers in applying for a grant have read and accept the Council's "Statement of Policy Containing Conditions and Terms Under Which Project Grants Are Made."

Checks payable to
Harvard Medical School

Mailing address for checks
Mr. Henry C. Meadow, 25 Shattuck Street
Boston, Massachusetts 02115

Principal investigator
Typed Name Emil R. Unanue, M.D.
Signature E. R. Unanue Date May 8, 1975
Telephone 617 (Area Code) 734-3300 (Number) 364 (Extension)

Responsible officer of institution
Typed Name Mr. Henry C. Meadow
Title Executive Secretary, Committee on Research and Development
Signature Henry C. Meadow Date 5.13.75
Telephone 617 (Area Code) 734-3300 (Number) 441 (Extension)

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Background Information

The literature on the functions of phagocytes (*i.e.*, monocyte-macrophages) in physiological and pathological states is extensive. It is not my intention to review it but to emphasize the major points relevant to this application having to do with the macrophage and its role in resistance and immunity. Macrophages participate to some degree in a wide number of processes, all of which deal with the inflammatory reaction against undesired materials. These cells are endowed with a number of properties that allow them to have a central role in inflammation (1): 1) macrophages distribute widely throughout various tissues; all the evidence indicates that they originate from a rapidly proliferating precursor found abundantly in bone marrow; from this precursor phagocytes differentiate and home to sites of inflammation—the differentiation and homing is under some control which has as yet to be defined in precise terms. 2) Phagocytes have membrane properties that enable them to bind a large number of materials; in particular, phagocytes take up a wide number of antigen molecules via "nonspecific" sites (*i.e.*, not characterized surface components) and by surface receptors for the Fc portion of Ig and for activated C3; following uptake, phagocytes interiorize and effectively degrade most of the foreign material (3). Hence, phagocytes play an essential role in elimination of antigens. Lastly, 3) phagocytes respond to environmental stimuli by becoming "activated"; activated phagocytes have increased biosynthesis of enzymes, are more active in phagocytosis, and are more microbicidal (4). (The term "activated" is a very poor one, meaning different things to different investigators. I used it to denote the macrophages that increase their metabolic function in response to external stimulation.) As a result of these three main properties, phagocytes

represent a pivotal cell in the inflammatory process and in immunity. *In inflammation*—in general—phagocytes are believed to play a major role in elimination of dead material and in wound healing. *In immune induction*, the role of phagocytes is of considerable importance. These cells, by handling and focusing antigen molecules play a crucial regulatory role in induction in part determining the size of the immunogenic stimulus (5). The uptake of antigen by macrophages represents a crucial step in the initiation of a full immune response. *In the efferent arc of immunity*, phagocytes represent the main cellular component of cellular type of immunities. Macrophages undoubtedly represent the major cellular component involved in resistance to certain infectious diseases, such as those produced by facultative intracellular bacterias (4). Their role in the processes is essential. A great unanswered question concerns the role of macrophages in general resistance to tumors. Two sets of observations point to some kind of a role, although this is still to be determined: 1) administration of certain live bacteria at the site of a tumor produces a marked infiltration with macrophages and a reduction of tumor growth—evidence would indicate that this process cannot be explained only by specific anti-tumor immunity (6); 2) in *in vitro* situations, macrophages have been found to exercise a cytostatic or cytocidal effect on tumors. These two series of observations suggest some kind of control of macrophages on cellular growth (7).

The exact manner by which macrophages exert their different functions is not clear. First, it is obvious that one set of effects is directly related to their capacity for endocytosis and intracellular elimination of foreign materials. A wealth of information is available on this process, which I will not detail since it is not the intention of this proposal to

study it. Very recently, however, it has become apparent that macrophages exert some biological control by secreting certain molecules into the extracellular environment. This function of macrophages was first indicated by *in vitro* experiments in which some lysosomal enzymes were found to be released into the outside medium following phagocytosis, suggesting to some that this release could be of significance in producing tissue damage (8). The process of secretion became, however, very evident for certain enzymes or bactericidal molecules, such as a plasminogen activator-like molecule or lysozyme (9, 10). These two molecules, in contrast to lysosomal enzymes, were destined mainly for export, *secretion*, and were not retained by the macrophage for intracellular handling, as it happened with lysosomal enzymes.

Our laboratory has been interested in the pathophysiology of macrophages mainly as it concerned the role of these cells in immune reactions. It is very obvious in a number of experimental systems—*in vivo* and *in vitro*—that macrophages exert an important regulatory role in immune induction. This role has been explained, in part, by the capacity of these cells to concentrate antigen and present a small finite number of undegraded molecules to the lymphocyte. In other words, macrophages apparently function as an antigen-focusing cell favoring the interaction of various collaborating lymphocytes with antigen. This helper function contrasts markedly with the phenomena observed *in vitro* in which macrophages stop the growth of tumor cells. Furthermore, it has been reported that macrophages may also play a detrimental effect on growth of non-neoplastic cells, including lymphocytes.

During the past year we set up a series of experiments to reappraise the effects of macrophages on various cells in culture. Our first experimental

design, which proved to be successful, was simply to culture macrophages under various conditions, *i.e.*, normal or activated, for various periods of time, following or not phagocytosis, etc., and then to test the culture fluids for their effects on other cells. It became immediately obvious that a number of active molecules were being secreted, some of which had contrasting effects on cellular growth. More importantly it appeared in preliminary experiments that the secretion of these molecules was regulated by the activity of the macrophages. The *in vitro* effects of the secreted material were quite dramatic. Indeed, their biological potential cannot be underestimated.

The first molecule to be found was a low-molecular-weight compound (about 600 daltons) that inhibited protein and DNA synthesis of various kinds of cells (11). The inhibitor was best seen in high-density cultures of macrophages. A number of cells, including neoplastic ones, cultured in medium containing the inhibitor did not synthesize DNA but were viable for at least 24 hours. The inhibitor was synthesized by the macrophages in culture, became bound to target cells, inhibited not only tumor cells but also lymphocyte proliferation and differentiation. This inhibitor was secreted only by macrophages; it was not found in cultures of lymphocytes, fibroblasts, or other cells. We have as yet no chemical definition of it. Preparative work has been in progress with Professor Manfred Karnovsky in the Department of Biochemistry at this Medical School. The important question of the relationship between its secretion and the activity of the macrophage has not been resolved yet. It is clear, however, that the material is found in cultures of both normal macrophages and macrophages activated by *Listeria* infection. My thoughts are that this molecule could conceivably play a biological role only in conditions where a large number

of macrophages focus around target cells, such as it occurs in immune granulomas. If not, it is difficult to visualize a role because of its very small size. Nevertheless, the biochemistry and mode of action are a fascinating biological problem. The presence of this inhibitor explains some of the cytostatic effects of macrophages on tumor cells seen in culture. It may also explain some of the results seen *in vivo* when certain adjuvants like BCG are injected with tumors, producing a marked macrophage infiltration and a reduction of tumor growth. Studies on this molecule are in progress.

The second set of molecules were found following removal by dialysis of the low-molecular-weight inhibitor (12, 13). Indeed, following dialysis (or in undialyzed medium from low-density cultures), we found molecule(s) that stimulated lymphocyte growth and differentiation. The biological activity of these sets of molecules were shown on several assay systems, all *in vitro*: 1) thymocytes were stimulated to proliferate and to respond to mitogens; 2) B lymphocytes, the precursor of antibody-forming cells, were stimulated to differentiate into plasma cells; 3) T lymphocytes increased their helper activity. For example, a dramatic effect of these macrophage factors (abbreviated MCF) was seen in cultures of spleen cells from athymic mice which could be made to respond immunologically to antigen. We have submitted a paper, now in press in the Journal of Experimental Medicine, which I enclose and which summarizes these points. This paper contains the basic methodology and the results on which this proposal is grounded. Of great interest are the following observations: 1) in the preliminary experiments, it was found that phagocytosis of particles *stimulated secretion* of twenty to one-hundred times more material; 2) in the only experiment done, chemical fractionation of the material suggests that there is more

than one molecule involved: the thymocyte stimulatory molecule is about 16,000 daltons, while the activity that stimulates B and T cells falls both into the 15,000 range as well as into the range of 100,000.

At the same time these studies were conducted, we decided to search also in macrophage culture fluids for activities that would attract cells to macrophages. It was our thought that if macrophages play a role in inductive events by antigen presentation as well as in resistance to infection, then certain mechanisms should be operative in bringing lymphoid, as well as other cells to foci of macrophages where antigen was being concentrated. Suggestive evidence to a relationship between antigen trapping by macrophages and lymphoid cell accumulation came from observations that the earliest cellular reaction to the entrance of antigen into a lymph node is an accumulation of lymphocytes. Indeed, radiolabeled lymphocytes accumulate into a node when particulate materials enter and are trapped by macrophages (14). Our early studies tested whether macrophage culture fluids obtained before or after phagocytosis would contain a molecule that attracted lymphocytes. We did the experiment in the rat, the assay for chemotaxis being done by Dr. Peter Ward of the University of Connecticut. We found that macrophage culture fluid *after phagocytosis* did contain a powerful chemotactic agent for lymphocytes. The importance of this observation, if true, is obvious since it places the macrophage at the time of phagocytosis in a central position regulating cell traffic.

Experimental Protocol

The purpose of this request is to further extend our analysis of biologically active molecules secreted by macrophages. At the present time we do not know how many different molecules are involved and are

being secreted, nor their biochemistry and precise mode of action. We have no idea of their role in *in vivo* processes nor do we know the mechanism controlling their biosynthesis and secretion. The long-range goals are to identify the secreted molecules in biochemical and biological terms. I now outline projects that represent a continuation of the present experiments: investigate: 1) the relationship between activity of macrophages and secretion of active products; 2) the biochemistry of the secreted molecules; and 3) their biological characterization.

Most technical details are included in the enclosed reprint (No. 3), which details our rationale for using the different assay systems of antibody formation. The basic technique is to obtain culture fluids from macrophages. These culture fluids at various dilutions are tested for their effects: 1) in stimulating DNA synthesis of thymocytes; 2) in increasing the helper activity of T lymphocytes in a hapten-carrier system *in vitro*; this is done by culturing spleen cells from selected mice immune to a hapten protein, in our case fluorescein (F) conjugated to hemocyanin (KLH), with the same antigen; after four days, the number of antibody-forming cells made to F is determined by a Jerne plaque assay (the spleen of the immune mice will contain antibody-forming cell precursors—B cells—reactive to F and ready to respond if challenged with the antigen, provided that T lymphocytes with the carrier protein, KLH, come into the system as helper cells—this being the classical setup of B-T cell interaction but now being modulated by products of phagocytes). 3) In producing differentiation of B cells; antibody-forming cell precursors cultured in MCF in the absence of T helper cells differentiate to plasma cells; we tested this by cultured primed spleen cells to F-KLH in the presence of F in an unrelated carrier protein (rabbit gamma globulin); and 4) chemotaxis using modified Boyden chambers as per conventional methods. All these studies will be done on macrophage fluids following dialysis to remove the inhibitor. I do plan to include analysis of it in undialyzed fluids.

A) The first project questions whether macrophages stimulated in various ways synthesize and secrete different amounts (or classes) of molecules. I believe it is important to outline the conditions, cellular or humoral, that may modulate the phenomenon of secretion. By doing this we will be in a strong position to place the phenomenon in a better

perspective and perhaps obtain an idea of its true biological significance. It is known that some responses of the macrophages are under modulation by environmental influences—indeed, phagocytosis, for example, induces more synthesis of lysosomal enzymes; infections associated with cellular immunity, likewise, lead to macrophage activation in terms of increased bactericidal activity. [illegible] investigate [illegible] the relationship between activation [illegible] process.

The idea is to carry out the experiments in the mouse (and/or rat). Macrophages will be isolated and stimulated *in vitro* by exposing them to a series of materials—most of the materials selected will be those that are readily taken up by the cell; included are those that result in marked adjuvant type of effects. Included are simple materials that are readily taken up by phagocytosis, such as latex particles, antigen-antibody complexes made up of soluble protein, or particulate antigens (such as sheep erythrocytes), and various dead bacteria. Of importance is to consider certain bacteria known to produce marked stimulation of macrophages (and also of immune responses): tubercule bacillus, *Corynebacterium parvum* and *Listeria monocytogenes*; and nonbacterial adjuvants, such as beryllium salt. Depending on the results, we will try to obtain an idea if the reaction is modulated by the step of membrane-particle interaction prior to phagocytosis or by the phagocytic process itself or by the *nature* of the material. This can be done by varying size and nature of the phylogogenic material (for example, antigen-antibody complexes can be attached to the surface of the culture dish; the macrophages will not ingest them, but still their surface receptors will interact with them. In these conditions, is secretion stimulated?

In summary, macrophages are to be exposed to the various materials and cultured for various time periods; culture fluids are then removed and tested biologically. Morphological and cytochemical determinations (for [illegible] acid phosphatase and total cell protein) will also be determined.

Another variant of this experiment is to administer the phlogogenic materials *in vivo* and then to determine if the spleen or peritoneal macrophages secrete more of the biologically active products.

It is important to consider that there may be more than one stimuli needed to produce an effect. Perhaps macrophages need to be activated

first (as it results, for example, by a systemic bacterial infection in which bacterial products plus the immune reaction produces system activation of all phagocytes) and then challenged by a phagocytic event. These kinds of relationships are amenable to exploration using macrophages exposed *in vivo* and *in vitro* or both in combination. The use of macrophages from different organs, including lung and spleen, is to be considered.

An important point to analyze along these lines is the relationship between secretion and biosynthesis of the active products. This will be tested in cultured macrophages in which protein synthesis is stopped following treatment with the various protein synthesis inhibitors.

In essence, we first plan a series of experiments testing whether various stimuli signal the macrophage to make and/or secrete the modulatory molecules. The experiments should give us an indication whether this function is controlled by external stimuli and of some of its basic mechanisms.

B) A second important goal is the chemical isolation and characterization of the molecules. This will be attempted by preparative and analytical methods such as electrophoresis or column chromatography. (Figure 5 of the enclosed paper No. 3 shows our initial attempt.) We have sufficient experience to be able to carry out at least part of the initial biochemical work. Our first idea is to culture macrophages in medium devoid of fetal calf serum (see the experiment of Figure 5 for an analysis of this point), concentrate the fluids, and attempt separation of the molecules by Sephadex G200 or G100 filtration. Chemical analysis of the purified or enriched materials will be done using polyacrylamide gel electrophoresis; the experiments also call for the sensitivity of the material to various enzymes, including proteolytic ones. My laboratory

does collaborative work with that of Prof. Manfred Karnovsky in the Department of Biochemistry here. Dr. Karnovsky has a large interest in these matters involving biochemistry of phagocytic functions and in continuously advising us in these matters. The ultimate goal of precise analysis will obviously require his assistance or that of members of his department. Organs, including lung and spleen, is to be considered.

C) The third project concerns itself with the biological characterization of secreted molecules. Several lines of investigation are contemplated. The first series of experiments described in the attached manuscripts suggests that one molecule has the capacity to increase or develop thymic function—thymocytes proliferate and are able to respond to phytohemagglutinin (immature thymocytes respond poorly, if at all, to PHA). This suggestion is strengthened by the results shown with the spleen cells of athymic mice that strikingly respond immunologically when cultured with antigen in the macrophage fluids. It is possible that the undifferentiated stem cells of the nude athymic mouse are rapidly stimulated to differentiate into T helper cells. We plan to test whether the MCF promotes differentiation of thymocytes (and stem cells formed in spleens of athymic mice). This will be done by: a) culturing the cells in MCF and assaying cytochemically for content of several thymic alloantigens—the thymocyte, as it differentiates changes its surface macromolecules, the alloantigen θ decreases, H-2 antigen increases; b) assaying culture cells for these immunological functions of mature T cells, *i.e.*, graft-*versus*-host type of assays and capacity to help B cells for antibody formation. The possibility that the MCF, likewise, promotes B cell differentiation to plasma cells was suggested by results in which enriched B cell populations cultured in MCF differentiate to secreting cells. This experiment will be further explored—B cells from

macrophages immunized to two or three antigens (horse red cells, the hapten F, or DNP) will be isolated by preparative methods, cultured at various times in the presence of MCF, and assayed for antibody-forming cells at various time intervals. By doing this we should obtain an idea whether the MCF in the presence or absence of antigen and in the absence of thymic helper cells promotes differentiation to plasma cells and the time required to do it.

The studies of chemotactic material consists of tests for chemotaxis using modified Boyden chambers and following conventional methods. As target cells, we will employ macrophages, lymphocytes (as whole populations or as semipurified B or T), and neutrophils. We, therefore, hope to establish whether there is a single chemotactic material and its cellular specificity.

All the experiments so far detailed call for analysis of these powerful secreted molecules and their relationship to macrophage function. The crux of the matter, however, is whether these molecules are operative in *in vivo* or simply represent a laboratory curiosity. The point holds true actually for many kinds of mediators of inflammation and of immunological reactions described so far, and its solution is not an easy one to tackle.

I plan to approach this problem along two lines of investigation: 1) to try and develop an antibody to the molecules in question and by doing this use the antibody as a probe to determine whether the molecules are found *in vivo* or whether the antibodies will modify particular macrophage functions. Making antibodies to soluble mediators has been very difficult in the past because most of the mediators are poor immunogens, apart from the fact that biochemical purity has not been accomplished. One hopeful point in favor of success in our experiments is that the macrophage

stimulatory molecules are found in culture fluids basically free of extraneous materials such as fetal calf serum. Our idea is to obtain the "purified" fractions from the experiments detailed in Project 2 and immunize rabbits repeatedly. I plan to follow conventional approaches, trying to use as pure a material as possible and doing the series of immunization gimmicks familiar to immunologists (*i.e.*, incorporation of the antigen into adjuvants like Freunds and/or attaching the antigen to a schlepper carrier). The antiserum will then be tested for its capacity to neutralize the activity *in vitro* before proceeding to any *in vivo* analysis of its effects.

2) The second approach is to test the MCF for the biological activity directly *in vivo*. That is to say, if these factors are also operative *in vivo*, one would expect that simple introduction of antigens with them should modify a regular immune response. Experiments testing conventional immune responses to antigen given with or without the MCF are thus contemplated. In particular, we are keen in testing athymic mice. We know from the *in vitro* data that spleen cells from these mice are made to respond to the MCF. Hence, the nude athymic mice may become the best host for examining this problem.

In summary, based on our initial experiments we know that macrophages secrete into the extracellular milieu a number of molecules having powerful modulating activity on various cells including lymphocytes. Our hypothesis is that the initial step in immunity involves the uptake of antigen by the phagocytes and from there the whole series of inductive events rapidly ensues, comprising a series of amplification steps and control mechanisms. Involved among the amplification steps may be the series of molecules described herein, all having as a function to trigger best the lymphocyte

which is the specific arm of the response. This proposal, then, represents an effort to understand further macrophage function and its role in local and generalized resistance and immunity. We should not overlook the potential use of these molecules in future clinical trials.

References

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2. Volkman, A., and Gowans, J. L. Brit. J. Exp. Path., *46*:50, 1965.
3. Cohn, Z. A. Adv. Immunol., *9*:97, 1968.
4. Mackaness, G. B. J. Exp. Med., *129*:973, 1969.
5. Unanue, E. R. Adv. Immunol., *15*:95, 1972.
6. Reviewed in R. C. Bast, *et al.* N. E. J. Med., *290*:1458, 1974.
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11. Calderon, J., Williams, R. T., and Unanue, E. R. Proc. Nat. Acad. Sci. USA, *71*:4273, 1974.
12. Calderon, J., and Unanue, E. R. Nature (Washington), *253*:359, 1975.
13. Calderon, J., Kiely, J.-M., Lefko, J. L., and Unanue, E. R. J. Exp. Med., in press, 1975.
14. Zatz, M. M., and Lance, E. M. J. Exp. Med., *134*:224, 1971.

Curriculum Vitae—Emil Raphael Unanue

Born: REDACTED

	B.S., Institute of Secondary Education
	M.D., University of Havana School of Medicine
1961 to 1962	Intern in Pathology, Presbyterian University Hospital, Pittsburgh, Pennsylvania
1962 to 1965	Research Fellow, Department of Experimental Pathology, Scripps Clinic and Research Foundation, La Jolla, California
1966 to 1968	Research Fellow, Immunology Division, National Institute for Medical Research, London, England
1968 to 1970	Associate, Department of Experimental Pathology, Scripps Clinic and Research Foundation, La Jolla, California
1970 to 1971	Assistant Professor of Pathology, Harvard Medical School, Boston, Massachusetts
1972 to 1974	Associate Professor of Pathology, Harvard Medical School, Boston, Massachusetts
1974	Mallinckrodt Professor of Immunopathology, Harvard Medical School, Boston, Massachusetts

Memberships

1966	REDACTED
1966	
1967	
1974	REDACTED

Honors and Award

1962	Recipient, Certificate of Education Council for Foreign Medical Graduates, U. S. A.
1966 to 1969	Fellow of Helen Hay Whitney Foundation
1968	Recipient, T. Duckett Jones Award of Helen Hay Whitney Foundation
1969 to 1970	Senior Fellow of the American Cancer Society, California Division
1971	Recipient, Research Career Developmental Award, National Institutes of Health
1973	Parke Davis Award, American Society for Experimental Pathology

Other Academic Activities

1972 Associate Editor, Journal of Immunology
1972 Associate Editor, Clinical Immunology and Immunopathology
1973 Associate Editor, International Archives of Allergy and Applied Immunology
1973 Member, Pathology A Study Section, National Institutes of Health
1974 Associate Editor, Journal of the Reticuloendothelial Society

Total publications: 96

Representative papers:

1. Unanue, E. R., and Cerottini, J.-C. The immunogenicity of antigen bound to the plasma membrane of macrophages. J. Exp. Med., *131*: 711, 1970.

2. Cruchaud, A., and Unanue, E. R. Fate and immunogenicity of antigens endocytosed by macrophages: a study using foreign red cells and immunoglobulin G. J. Immunol., *107*:1329, 1971.

3. Unanue, E. R. The regulatory role of macrophages in antigenic stimulation. Adv. Immunol., *15*:95, 1972 (summarizes all our work with macrophages to 1972).

4. Lane, F. C., and Unanue, E. R. Requirement of T (thymus) lymphocytes for resistance to listeriosis. J. Exp. Med., *135*:1104, 1972.

5. Katz, D. H., and Unanue, E. R. Critical role of determinant presentation in the induction of specific responses in immunocompetent lymphocytes. J. Exp. Med., *137*:967, 1973.

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Representative papers, continued

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9. Calderon, J., Williams, R. T., and Unanue, E. R. An inhibitor of cell proliferation released by cultures of macrophages. Proc. Nat. Acad. Sci. USA, *71*:4273, 1974 (enclosed as Reprint No. 1).

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Curriculum Vitae

Miguel J. Stadecker

Born: REDACTED

Family: REDACTED

Education:

R B.S., National School Nr. 8, Buenos Aires, Argentina

R M.D., University of Buenos Aires School of Medicine, Buenos Aires, Argentina

1967-1970: Instructor in Pathology, University of Buenos Aires School of Medicine, Buenos Aires, Argentina

Assistant Pathologist, Ramos Mejia Hospital, Buenos Aires, Argentina

1970-1971: Second Assistant Resident, Department of Pathology, Massachusetts General Hospital, Boston, Massachusetts

1971-1972: Third Assistant Resident, Department of Pathology, New England Medical Center Hospital, Boston, Massachusetts

Instructor in Pathology, Tufts University School of Medicine, Boston, Massachusetts

1972-1973: Chief Resident, Pathology, New England Medical Center Hospital, Boston, Massachusetts

Instructor in Pathology, Tufts University School of Medicine, Boston, Massachusetts

Graduate Student in Immunology, Tufts University School of Medicine, Boston, Massachusetts

1973-1974: Fellow in Immunology-Pathology, Tufts University School of Medicine, Boston, Massachusetts

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